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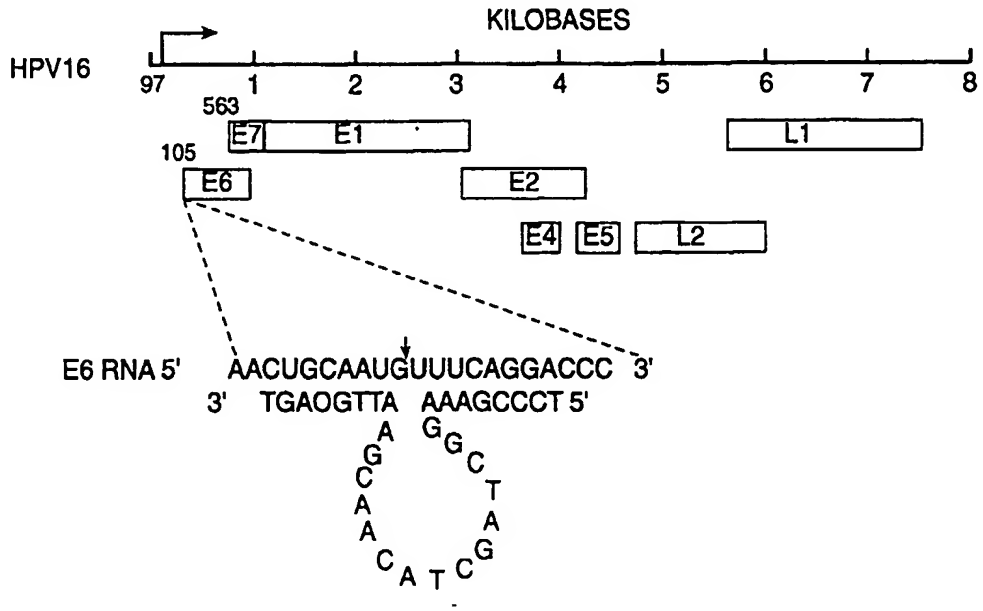

(57) Abstract

This application provides a DNAzyme which specifically cleaves human papilloma viral (HPV) mRNA, comprising a 15-nucleotide catalytic domain and two binding domains, one binding domain contiguous with the 5' end of the catalytic domain and the other binding domain contiguous with the 3' end of the catalytic domain. This invention also provides a pharmaceutical composition for inhibiting the onset of, or treating, an HPV-related disorder, which comprise the instant DNAzyme and a pharmaceutically acceptable carrier suitable for topical administration. Finally, this invention provides methods of using the instant pharmaceutical composition to inhibit the onset of, and treat, HPV-related disorders.

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DNAZYMES AND METHODS FOR TREATING HPV-RELATED DISORDERS

Field of the Invention

This invention relates to using DNAzymes to treat and inhibit the onset of human papilloma virus-related disorders. The DNAzymes accomplish this end by cleaving human papilloma viral mRNA, whose expression in human host cells is required for related disorders to occur.

Background of the Invention

Clinical Aspects of HPV

Human papilloma viruses, also referred to herein as "HPV's", are the most common sexually transmitted viral agents in the United States. HPV infects from 5% to 20% of persons aged 15 to 49 (Nuovo). HPV infections occur in both men and women, and at sites such as the genital area, oral cavity, hands, feet and skin.

Genital HPV infection first results in the formation of genital warts. The Center for Disease Control and Prevention has estimated that nearly 1 million new cases of HPV-mediated genital warts are diagnosed every year in the United States alone.

HPV infection is often co-existent with disorders such as syphilis, gonorrhea, chlamydia, herpes simplex virus ("HSV"), and human immunodeficiency virus ("HIV"). Moreover, HPV infection is common among all races and socioeconomic groups, and is prevalent throughout the world among sexually active people.

As mentioned already, HPV infection first manifests itself in the form of genital warts. Unfortunately, however, the infection can later result in additional and far more severe disorders. Specifically, among infected women, HPV is currently the central risk factor for cervical neoplasia and cervical cancer. Cervical cancer remains the second most common cancer among women worldwide. Over 80% of such cases can be attributed to HPV of a limited number of HPV types (as discussed in more detail below). HPV 16, 18, 31 and 45 combined account for over two-thirds of the viral types identified in cervical cancer specimens. The risk of disease progression following HPV infection seems to be related to the persistence of the infection, HPV viral type and viral load.

Because most cases of cervical dysplasia and carcinoma are associated with HPV infection, the increasing prevalence of genital warts in young women may portend a future increase in the rate of resulting cervical dysplasia and carcinoma.

HPV Structure and Biology

HPV's are small naked viruses with an icosahedral symmetry, 72 capsomers, and a double-stranded circular DNA genome. The viral DNA is complexed with low-molecular weight histones of cellular origin. The genome consists of a double-stranded DNA molecule of approximately 8,000 base pairs, having a molecular weight of 5×10^6 daltons. All of the genome's open-reading frames ("ORF's") are located on one strand.

HPV types are numerous (Phelps; Shillitoe), and examples of predominant types are provided in the Examples section below. All HPV types have a similar genetic organization (Figure 1). The viral genome is divided into three regions: (a) an early region (about 4.5 kb) necessary for transformation; (b) a late region (about 2.5 kb) encoding the capsid proteins; and (c) a regulatory region (about 1 kb) containing the origin of replication and many control elements for transcription and replication (zur Hausen).

HPV gene expression is controlled by both viral and cellular factors. The regulatory region in the HPV genome is called the upstream regulatory region ("URR"). Transcriptional enhancers are present in the central part of the URR (Shillitoe). These enhancers are composed of a number of sequence modules of low or moderate activity. These modules act cooperatively as a single, strong enhancer whose activity depends on interactions with cellular transcriptional factors such as *jun/fos*, NF-1 (nuclear factor-1), Oct-1 (octamer-binding factor-1), and TEF (transcriptional enhancing factor). A similar set of binding sites for these cellular proteins is generally found in the center of the URR of genital HPV's. Both AP1 (activator protein-1) and NF1 recognition sites are consistent features of all enhancers. The main enhancer in HPV's is keratinocyte-specific, which may help explain the tissue tropism of these viruses (Dollard).

Many HPV genomes encode three transforming proteins: E7, E6 and E5. It was known early on that cervical carcinomas contain HPV genomes which, although disrupted and rearranged as a consequence of

integration, uniformly express the E6 and E7 genes. This uniform expression is in contrast to the expression of several other early viral genes such as E1, E2, E4 and E5, which are often separated from the viral promoter, deleted, or significantly rearranged. The consistent retention and expression of the E6 and E7 genes in tumors suggest that these genes are also required for the maintenance of the malignant state, and not merely for its development.

The HPV E7 protein is an acidic phosphoprotein consisting of approximately 100 amino acid residues. While initial studies localized the E7 protein predominantly to the cytoplasm, subsequent immunofluorescence and cell fractional assays have revealed that E7 also resides within the nuclear compartment (Sato).

The E7 protein binds to the retinoblastoma-susceptibility gene product ("RB") (Dyson). The RB protein is a phosphoprotein which, in its under-phosphorylated form, appears to negatively regulate entry into the S-phase of the cell cycle. Initiation of the S-phase is accompanied by the specific phosphorylation of the RB protein via cyclin-dependent kinases ("cdks"). When cells enter mitosis, the RB protein is dephosphorylated and thus returned to its growth-inhibitory form.

The E7 protein binds with the highest affinity to the under-phosphorylated form of RB, thereby interfering with a critical growth regulatory step in the cell cycle. Since the RB protein associates not only with HPV E7 protein but with several cell proteins as well (including the E2F transcriptional factor, cdk p34 cdc2, and phosphatase 1A), it is

likely that E7 also modulates several of these interactions. Indeed, the binding of E7 to RB leads to dissociation of an E2F/RB complex. This complex, in turn, appears to inhibit transcription of genes possessing E2F-binding motifs (Morris).

The HPV E6 protein contains approximately 150 amino acids. From immunofluorescence microscopy, it is known that E6 proteins from HPV 16 and 18 reside in the nuclear matrix as well as in non-nuclear membrane fractions.

It was found that the E7 protein of HPV associates with the RB protein, as does E1A. Based on this finding, subsequent studies also showed that the HPV E6 protein associates with p53, as does E1B (Werness). The p53 protein is involved in cellular transformation by DNA tumor viruses, as indicated by its role as a target for the SV40 large T-antigen. Although p53 is a common target for DNA-transforming proteins, the consequences of this role are quite different. Specifically, the E6/p53 interaction results in the degradation of the p53 protein, unlike the interaction between p53 and the large T-antigen or E1B, which stabilizes the p53 protein (Scheffner). This degradation is ubiquitin-dependent and mediated through interaction with a 100-kD cellular protein known as "E6-AP". The degradation of p53 is mediated by the E6 protein of the high-risk HPV's (e.g. HPV 16 and 18), but not low-risk HPV's (e.g. HPV 6 and 11), suggesting an important role for this process in the development of malignancy.

The HPV E6 gene also possesses its own independent transforming activities, in addition to its role in augmenting E7-mediated transformation of

human genital keratinocytes,. The E6 genes of both high- and low-risk HPV types are able to facilitate cellular immortalization, although the high-risk E6 proteins appear to be the most efficient at doing so (Crook).

Present Treatment Strategies

Despite the prevalence of HPV infection and its association with malignant disease, there is no proven antiviral therapy for its treatment. The goal of current therapeutic strategies is the removal of exophytic warts and elimination of signs and symptoms, not the eradication of viral DNA. While nearly all genital warts can be eliminated by such treatments as cryotherapy, electrodesiccation, or surgical removal, recurrence rates are generally unacceptable. This high recurrence rate is probably due to the presence of virus particles in adjacent normal tissue.

DNAzymes

In human gene therapy, antisense nucleic acid technology has been one of the major tools of choice to inactivate genes whose expression causes disease and is thus undesirable. The anti-sense approach employs a nucleic acid molecule that is complementary to, and thereby hybridizes with, an mRNA molecule encoding an undesirable gene. Such hybridization leads to the inhibition of gene expression.

Anti-sense technology suffers from certain drawbacks. Anti-sense hybridization results in the formation of a DNA/target mRNA heteroduplex. This heteroduplex serves as a substrate for RNAse H-mediated

degradation of the target mRNA component. Here, the DNA anti-sense molecule serves in a passive manner, in that it merely facilitates the required cleavage by endogenous RNAse H enzyme. This dependence on RNAse H confers limitations on the design of anti-sense molecules regarding their chemistry and ability to form stable heteroduplexes with their target mRNA's. Anti-sense DNA molecules also suffer from problems associated with non-specific activity and, at higher concentrations, even toxicity.

As an alternative to anti-sense molecules, catalytic nucleic acid molecules have shown promise as therapeutic agents for suppressing gene expression, and are widely discussed in the literature (Haseloff; Breaker (1994); Koizumi; Otsuka; Kashani-Sabet; Raillard; and Carmi). Thus, unlike a conventional anti-sense molecule, a catalytic nucleic acid molecule functions by actually cleaving its target mRNA molecule instead of merely binding to it. Catalytic nucleic acid molecules can only cleave a target nucleic acid sequence if that target sequence meets certain minimum requirements. The target sequence must be complementary to the hybridizing regions of the catalytic nucleic acid, and the target must contain a specific sequence at the site of cleavage.

Catalytic RNA molecules ("ribozymes") are well documented (Haseloff; Symonds; and Sun), and have been shown to be capable of cleaving both RNA (Haseloff) and DNA (Raillard) molecules. Indeed, the development of in vitro selection and evolution techniques has made it possible to obtain novel ribozymes against a known substrate, using either random variants of a known ribozyme or random-sequence RNA as a starting point (Pan; Tsang; and

Breaker (1994)). Ribozymes, however, are highly susceptible to enzymatic hydrolysis within the cells where they are intended to perform their function. This in turn limits their pharmaceutical applications.

Recently, a new class of catalytic molecules called "DNAzymes" was created (Breaker (1995); Santoro). DNAzymes are single-stranded, and cleave both RNA (Breaker (1994); Santoro) and DNA (Carmi). A general model for the DNAzyme has been proposed, and is known as the "10-23" model. DNAzymes following the "10-23" model, also referred to simply as "10-23 DNAzymes", have a catalytic domain of 15 deoxyribonucleotides, flanked by two substrate-recognition domains of seven to nine deoxyribonucleotides each. In vitro analyses show that this type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions under physiological conditions (Santoro).

DNAzymes show promise as therapeutic agents. However, DNAzyme success against a disease caused by the presence of a known mRNA molecule is not predictable. This unpredictability is due, in part, to two factors. First, certain mRNA secondary structures can impede a DNAzyme's ability to bind to and cleave its target mRNA. Second, the uptake of a DNAzyme by cells expressing the target mRNA may not be efficient enough to permit therapeutically meaningful results. For these reasons, merely knowing of a disease and its causative target mRNA sequence does not alone allow one to reasonably predict the therapeutic success of a DNAzyme against that target mRNA, absent an inventive step.

Summary of the Invention

This invention provides a DNAzyme which specifically cleaves HPV mRNA, comprising

(a) a catalytic domain that has the nucleotide sequence GGCTAGCTACAACGA and cleaves mRNA at any purine:pyrimidine cleavage site at which it is directed,

(b) a binding domain contiguous with the 5′ end of the catalytic domain, and

(c) another binding domain contiguous with the 3′ end of the catalytic domain,

wherein the binding domains are complementary to, and therefore hybridize with, the two regions immediately flanking the purine residue of the cleavage site within the HPV mRNA, respectively, at which DNAzyme-catalyzed cleavage is desired, and wherein each binding domain is at least six nucleotides in length, and both binding domains have a combined total length of at least 14 nucleotides.

This invention also provides a pharmaceutical composition for treating or inhibiting the onset of an HPV-related disorder, which comprises the instant DNAzyme and a pharmaceutically acceptable carrier suitable for topical administration.

This invention further provides a method for inhibiting the onset of an HPV-related disorder in a subject at risk of contracting the disorder, which comprises topically administering to the subject a prophylactically effective dose of the instant pharmaceutical composition.

Finally, this invention provides a method for treating an HPV-related disorder in a subject, which comprises topically administering to the subject a therapeutically effective dose of the instant pharmaceutical composition.

Brief Description of the Figures

Figure 1 shows the HPV 16 genomic structure and related targeting strategy. "10-23" model-based DNAzymes were designed against the HPV 16 E6 gene at the AUG start codon region.

Figure 2 shows a kinetics study of anti-HPV 16 E6 DNAzymes. A short synthetic RNA substrate oligonucleotide (final concentration 1μM) was end-labeled with [γ-^{32}P]ATP at 37°C for 30 minutes and 75°C for 2 minutes. Single turnover kinetics was achieved in a reaction mixture containing an 8-fold excess of DNAzyme over substrate.

Figure 3 shows the cleavage activity of modified anti-E6 DNAzymes. DNAzymes were chemically modified as indicated. Cleavage reactions were performed in the presence of 10 mM $MgCl_2$, 50 mM Tris.Cl, pH 7.5, at 37°C for 60 minutes.

Figure 4 shows the stability of the modified DNAzymes in human serum. 150 mM DNAzyme was incubated in 100% human serum (Sigma). 5 μl of sample were withdrawn at various time points, and the DNAzyme was labeled with [γ-^{32}P]ATP and assayed on a 16% PAGE.

Figure 5 shows a schematic description of a transient assay for DNAzyme-mediated down-regulation of the E6 gene.

Figure 6 shows the inhibition of E6 gene expression in a transient assay. After co-transfection of 10 μM DNAzyme and 2.5 μg E6 expression vector into 3T3 cells, RNA samples were prepared from the cells,

blotted onto membranes and hybridized with ^{32}P-labelled E6 probe. The signals were analyzed using a PhosphorImager.

Detailed Description of the Invention

This invention is directed to using DNAzymes to treat and inhibit the onset of human papilloma virus-related disorders. Once human papilloma virus, or HPV, infects a subject, this infection manifests itself as genital warts. These warts often precede more serious HPV-related disorders such as cervical dysplasia and carcinoma. The expression of HPV genes, via mRNA synthesis, is vital to HPV's ability to infect and propagate disease in its human host. Such mRNA expression is the "Achilles heel" at which this invention is directed, in that the invention employs DNAzymes which specifically cleave HPV mRNA and thereby prevent HPV gene expression.

More specifically, this application provides a DNAzyme which specifically cleaves HPV mRNA, comprising

(a) a catalytic domain that has the nucleotide sequence GGCTAGCTACAACGA and cleaves mRNA at any purine:pyrimidine cleavage site at which it is directed,

(b) a binding domain contiguous with the 5' end of the catalytic domain, and

(c) another binding domain contiguous with the 3' end of the catalytic domain,

wherein the binding domains are complementary to, and therefore hybridize with, the two regions immediately flanking the purine residue of the cleavage site within the HPV mRNA, respectively, at which DNAzyme-catalyzed cleavage is desired, and wherein each binding domain is at least six nucleotides in length, and both binding domains have a combined total length of at least 14 nucleotides.

As used herein, "DNAzyme" means a DNA molecule that specifically recognizes and cleaves a distinct target nucleic acid sequence, which can be either DNA or RNA. The instant DNAzyme cleaves RNA molecules, and is of the "10-23" model, as shown in Figure 1, named so for historical reasons. This type of DNAzyme is described in Santoro. The RNA target sequence requirement for the 10-23 DNAzyme is any RNA sequence consisting of NNNNNNNR*YNNNNNN, NNNNNNNNR*YNNNNN or NNNNNNR*YNNNNNNN, where R*Y is the cleavage site, R is A or G, Y is U or C, and N is any of G, U, C, or A.

Within the parameters of this invention, the binding domain lengths (also referred to herein as "arm lengths") can be of any permutation, and can be the same or different. Various permutations such as 7+7, 8+8 and 9+9 are envisioned, and are exemplified more fully in the Examples that follow. It is well established that the greater the binding domain length, the more tightly it will bind to its complementary mRNA sequence. Accordingly, in the preferred embodiment, each binding domain is nine nucleotides in length. In one embodiment, the instant DNAzyme has the sequence TCCCGAAAGGCTAGCTACAACGAATTGCAGT (Figure 1).

In applying DNAzyme-based treatments, it is important that the DNAzymes be as stable as possible against degradation in the intra-cellular milieu. One means of accomplishing this is by incorporating a 3'-3' inversion at the 3' terminus of the DNAzyme. More specifically, a 3'-3' inversion (also referred to herein simply as an "inversion") means the covalent phosphate bonding between the 3' carbons of the 3' terminal nucleotide and its adjacent nucleotide. This type of bonding is opposed to the normal phosphate bonding between the 3' and 5' carbons of adjacent

nucleotides, hence the term "inversion." Accordingly, in the preferred embodiment, the 3'-end nucleotide residue is inverted in the binding domain contiguous with the 3' end of the catalytic domain. In addition to inversions, the instant DNAzymes can contain modified nucleotides. Modified nucleotides include, for example, N3'-P5' phosphoramidate linkages, and peptide-nucleic acid linkages. These are well known in the art (Wagner).

In this invention, any contiguous purine: pyrimidine nucleotide pair within the HPV mRNA can serve as a cleavage site. In the preferred embodiment, purine:uracil is the desired purine:pyrimidine cleavage site.

The HPV mRNA region containing the cleavage site can be any region. For example, the location within the mRNA at which DNAzyme-catalyzed cleavage is desired can be the translation initiation site, a splice recognition site, the 5' untranslated region, or the 3' untranslated region. In one embodiment, the cleavage site is located at the translation initiation site.

The sequences of HPV mRNA's, and/or DNA encoding same, are well known (Myers). As used herein, "HPV mRNA" means any mRNA sequence encoded by an HPV strain, such as HPV 16. Examples of such mRNA's include those encoding HPV 16 proteins E5, E6 and E7. In the preferred embodiment, the HPV mRNA to which the instant DNAzyme is directed encodes proteins E6 or E7. HPV mRNA's include both mature as well as immature mRNA's. Within the parameters of this invention, determining the HPV mRNA cleavage site, the required sequences of each binding region, and thus the sequence of then

entire DNAzyme, can be done according to well known methods.

This invention also provides a pharmaceutical composition for treating or inhibiting the onset of an HPV-related disorder, which comprises the instant DNAzyme and a pharmaceutically acceptable carrier suitable for topical administration.

As used herein, the term "HPV-related disorder" means any disease or physiological abnormality resulting from HPV infection. In one embodiment, the HPV-related disorder is cervical carcinoma, cervical dysplasia or genital warts. Other HPV-related disorders are exemplified in the Examples section below.

In this invention, topically administering the instant pharmaceutical compositions can be effected or performed using any of the various methods and delivery systems known to those skilled in the art. The topical administration can be performed, for example, transdermally, orally, transmucosally, and via topical injection.

Pharmaceutical carriers for topical administration are well known in the art, as are methods for combining same with active agents to be delivered. Examples of topical carriers and their uses are well known in the art (Ramchandani; Barry; Wenniger; Martindale's Parmacopoeia; U.S. Pharmacopeoia). The following delivery systems, which employ a number of routinely used carriers, are only representative of the many embodiments envisioned for administering the instant composition.

Dermal delivery systems include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In the preferred embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,N^{I},N^{II},N^{III}-tetramethyl-N,N^{I},N^{II},N^{III}-tetrapalmityl-spermine and dioleoyl phosphatidylethanolamine (DOPE)(GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-trimethyl-ammoniummethylsulfate) (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

Transmucosal delivery systems include tablets, suppositories, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

Injectable drug delivery systems include solutions, suspensions, gels, microspheres and polymeric injectables, and can comprise excipients such as solubility-altering agents (e.g., ethanol, propylene

glycol and sucrose) and polymers (e.g., polycaprylactones and PLGA's).

Oral delivery systems include tablets and capsules. These can contain excipients such as binders (e.g., hydroxypropylmethylcellulose, polyvinyl pyrilodone, other cellulosic materials and starch), diluents (e.g., lactose and other sugars, starch, dicalcium phosphate and cellulosic materials), disintegrating agents (e.g., starch polymers and cellulosic materials) and lubricating agents (e.g., stearates and talc).

As used herein, "inhibiting" the onset of an HPV-related disorder means either lessening the severity of the disorder once HPV infection occurs, or preventing the onset of the HPV-related disorder entirely. In the preferred embodiment, inhibiting the onset of an HPV-related disorder means preventing its onset entirely. "Treating" an HPV-related disorder means either slowing, stopping or reversing the disorder's progression. In the preferred embodiment, "treating" an HPV-related disorder means reversing the disorder's progression, ideally to the point of eliminating the disorder itself.

This invention further provides a method for inhibiting the onset of an HPV-related disorder in a subject at risk of contracting the disorder, which comprises topically administering to the subject a prophylactically effective dose of the instant pharmaceutical composition. Subjects at risk of contracting HPV-related disorders include, for example, (a) those infected with high-risk HPV types; (b) those having abnormal PAP Smear results; (c) those afflicted

with cervical cancer; and (d) those who are immuno-compromised.

Determining a prophylactically effective dose of the instant pharmaceutical composition can be done based on animal data using routine computational methods. In one embodiment, the prophylactically effective dose contains between about 0.1 mg and about 1 g of the instant DNAzyme. In another embodiment, the prophylactically effective dose contains between about 1 mg and about 100 mg of the instant DNAzyme. In a further embodiment, the prophylactically effective dose contains between about 10 mg and about 50 mg of the instant DNAzyme. In yet a further embodiment, the prophylactically effective dose contains about 25 mg of the instant DNAzyme.

Finally, this invention provides a method for treating an HPV-related disorder in a subject, which comprises topically administering to the subject a therapeutically effective dose of the instant pharmaceutical composition.

Determining a therapeutically effective dose of the instant pharmaceutical composition can be done based on animal data using routine computational methods. In one embodiment, the therapeutically effective dose contains between about 0.1 mg and about 1 g of the instant DNAzyme. In another embodiment, the therapeutically effective dose contains between about 1 mg and about 100 mg of the instant DNAzyme. In a further embodiment, the therapeutically effective dose contains between about 10 mg and about 50 mg of the instant DNAzyme. In yet a further embodiment, the therapeutically effective dose contains about 25 mg of the instant DNAzyme.

This invention will be better understood by reference to the Examples which follow, but those skilled in the art will readily appreciate that they are only illustrative of the invention as described more fully in the claims which follow thereafter. In addition, various publications are cited throughout this application. The disclosures of these publications are hereby incorporated by reference into this application to describe more fully the state of the art to which this invention pertains.

Examples

Example 1
HPV-Related Disorders

Table 1
HPV-Related Disorders

Disorder	Prominent HPV-Types	Infection Site
Non-Malignant		
Cutaneous Warts	1-4, 10	hands, feet
Condylomata Acuminata	6, 11	genital
Laryngeal Papillomas	"	larynx
Malignant		
Cervical Cancer	16, 18, 31, 33, 35, 39, 45, 51, 56	cervix, vulva anus, penis
Anogenital Cancer	"	"
Intraepithelial Neoplasia	"	"
Oral Cancer	16, 18	oral cavity
Epidermodysplasia Verruciformis	5, 8	skin

Example 2
Design of DNAzymes against the HPV16 E6 gene

The oncogenic function of HPV has been assigned primarily to two early genes, E6 and E7. As discussed previously, these transforming genes are expressed in HPV-containing carcinomas and cell lines, and have transforming potential both in vitro and in vivo. Inhibition of E6 and E7 can be achieved by using antisense transcripts and oligonucleotides. This inhibition leads to reduced growth rates and

loss of the transformed phenotype of cervical and oral carcinoma cell lines (Cowsert; Hu; von Knebel).

Here, the HPV 16 E6 gene has been chosen as a target for DNAzyme action. Based on previous data, the region around the translation initiation site (AUG) of the E6 gene was chosen as the target sequence (Sun (1995a); Sun (1995b)) (Figure 1).

DNAzymes that were designed against the E6 gene are based on the "10-23" model (Santoro). A range of DNAzymes containing the 10-23 catalytic domain targeted against the translation initiation region of the HPV E6 gene were synthesized with various modifications and arm lengths as shown in Figure 1.

Example 3
In vitro characterization of anti-HPV 16 DNAzymes

Single turnover kinetics was achieved in a reaction containing an 8-fold excess of DNAzyme over substrate. Each DNAzyme was pre-equilibrated at 37°C for 10 minutes in a buffer containing 50 mM Tris.HCl, pH 7.5, 10 mM $MgCl_2$. The labeled substrate was also pre-equilibrated in the same buffer separately at 37°C for 10 minutes. At time zero, the solution containing the DNAzyme was mixed with the substrate (at final concentrations of 320 nM and 40 nM, respectively), and incubated at 37°C for t = 5, 10, 20, 30, 60 minutes. At each time point, 2 µl of the reaction were transferred to an equal volume of ice-cold stop buffer (90% formamide, 20 mM EDTA, loading dye).

The product and unreacted substrate were resolved by electrophoresis on a denaturing 16% polyacrylamide sequencing gel. The gel was then exposed to a phosphor-storage screen for scanning in a Molecular

Dynamics PhosphorImager. The extent of reaction for each time point was determined by densitometry using Image Quant software (Molecular Dynamics). The percentage of band intensity volume in the cleavage product was compared to that of the combined substrate, and product intensity was determined. The results were then analyzed graphically in a plot against time.

A line of best fit was generated for the data (least-squares) using the equation $\%P = \%P_\infty - C \cdot \exp[-kt]$, where %P is the percentage product, $\%P_\infty$ is the percentage product at $t = \infty$, C is the difference in %P between $t = \infty$ and $t=0$, and k is the first order rate constant. The first order rate constant (k_{obs}) was used to compare the rates of different DNAzymes.

A range of DNAzymes containing the 10-23 catalytic domain targeted to the HPV 16 E6 translation initiation site were synthesized with various chemical modifications and binding arm lengths. These DNAzymes were then compared by analyzing their single turnover kinetics on a short synthetic RNA substrate (Figure 2).

By ranking the activity of these DNAzymes on the basis of their observed rate constants (k_{obs}) (table 1), the best result was seen with the molecule containing 10-base arms, each modified by phosphorothioate linkages ($k_{obs} = 0.33\ min^{-1}$).

This result is surprising, considering that the kinetic activity of the 8-base arm version of this DNAzyme was severely impaired by the inclusion of phosphorothioate-modified linkages ($k_{obs} = 0.02\ min^{-1}$). However, a number of reports investigating the hybridization strength of oligonucleotides have shown

that the melting temperature for a DNA/RNA heteroduplex is significantly reduced when the DNA is phosphorothioate-modified. It is possible that the reduction in hybridization stability as a consequence of phosphorothioate modification was detrimental to catalysis by the short 8-base arm DNAzyme. The remarkable increase in activity in the longer 10-base version indicates that a duplex length-related increase in hybridization stability improves the observed rate of catalysis.

Other than the 10-base arm phosphorothioate DNAzyme, the most active DNAzymes are those with 8-base arms, either unmodified or modified at the 3′ terminal by an amine group. These molecules have observed first order rate constants of 0.23 and 0.21 min^{-1}, respectively. The activity of the amine-modified DNAzyme was not affected by the extension to the terminal phosphate.

The next most active molecules tested are the 9-base arm DNAzyme containing phosphorothioate linkages, the DNAzyme with 8-base arms protected by a 3'-3' inversion, and the unmodified DNAzyme with 7-base arms (k_{obs} = 0.18, 0.15, 0.07, respectively). The 7-base arm DNAzyme with a 3′-3′ inversion displayed a k_{obs} = 0.04. The decline in activity observed between the unmodified DNAzymes and those with 3′-3′ inversions indicates that the modified base might not participate in hybridization with the target RNA. However, this loss of stability, apparent from the drop in k_{obs}, was found to be recoverable by increasing the DNAzyme arm length.

Table 2
First order kinetics of HPV16 E6 DNAzymes

Arm length	K_{obs} (min^{-1}) Unmodified	3'-INV	3'-amine	PT*
7 + 7	0.07	0.04	-	-
8 + 8	0.21	0.15	0.23	0.02
9 + 9	-	-	-	0.18
10 + 10	-	-	-	0.33

* PT = Phosphorothioate. Full arms of DNAzymes were modified by phosphorothioate linkage.

Example 4
Chemical modification of HPV 16 DNAzymes and their stability in human serum

A range of chemically modified oligonucleotides were synthesized and tested in an in vitro cleavage assay. The DNAzyme with 8/8 arms targeting the E6 start codon was separately modified by (i) a 3′-3′-terminal base inversion, (ii) a 3′-phosphorpropylamine group, and (iii) 1-5 bp phosphorothioate linkages (i.e. "phosphorothioate cap") in the DNAzyme arms. All of these modifications maintained DNAzyme activity to varying degrees, with the 3′-inversion showing the greatest ability to preserve activity, followed by the phosphorpropylamine modification, and the single base phosphorothioate cap (Figure 3).

DNAzyme stability was tested in 100% human serum. The experiment was done using 10 µM unlabelled DNAzyme in 1 ml 100% human serum incubated at 37°C. Duplicate 5µl samples were removed at the time points of 0, 2, 8, 24, 48, and 96 hours for DNAzymes 41 (7/7 arms

unmodified), 42 (7/7 arms, 3'-inversion), 23 (7/7 arms, 3'-amine), 24 (7/7 arms, 1'-phosphorothioate caps), 26 (7/7 arms, 3'-phosphorothioate caps) and 28 (7/7 arms, 5'-phosphorothioate caps). Immediately upon sampling, 295 µl TE were added to the 5µl aliquot, and phenol/chloroform extraction was performed. All the samples from each time point were end-labeled with ^{32}P and run directly on 16% PAGE gels without further purification or precipitation. This procedure ensured that all intact DNAzymes and degradation products were detectable. The results of this experiment indicate that DNAzyme 42 was the most stable, followed by DNAzymes 23, 28, 26, 24 and 41. The half-life of the unmodified DNAzyme is less than 8 hours under these conditions (Figure 4).

Example 5
Biological assays for HPV 16 E6/E7-cleaving DNAzymes

(a) E6 RNA expression assays

A transient assay system was used for anti-HPV DNAzyme testing (Figure 5). This assay is based on co-transfection of an HPV 16 E6 expression vector and DNAzymes into 3T3 cells to measure potential DNAzyme-mediated suppression of target RNA.

Briefly, 3T3 cells were plated at $3x10^5$ cells per well in 6-well plates. The following day, transfections were performed using DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-trimethyl-ammoniummethylsulfate) mixed with 2-5 µg of plasmid DNA (pCDNA3.E6) and 10 mM DNAzyme or control oligonucleotides. After 17 hours of incubation, the cells were washed, trypsinised and resuspended. RNA was then extracted using a Qiagen RNeasy kit (Qiagen). Typically, approximately 15 µg of RNA was obtained per sample. One part RNA (2µg) was then added to three parts of formamide/formaldehyde

buffer and heated to 65°C for 5 minutes. The mixture was placed on ice, and an equal part of 20x SSC buffer was added. The RNA samples were then blotted on to a Hybond membrane pre-soaked in 10x SSC with a Bio-Rad dot-blot apparatus. The blot was then hybridized with radio-labeled E6 probe, and analyzed using a PhosphorImager.

These assays were conducted to compare the efficacy of DNAzymes 41 and 42 with that of random control DNAzyme 49 at inhibiting the expression of E6. As shown in Figure 6, DNAzymes 41 and 42 proved effective at inhibiting the expression of E6, when compared with DNAzyme 49.

(b) Cell cycle assay on DNAzyme-treated HPV 16-positive CasKi cells

10 mM anti-E6 DNAzyme Dz22 and control oligonucleotide were incubated with CasKi cells for 24 hours before being subjected to FACS analysis. Results showed that the DNAzyme increases the proportion of G1 cells, suggesting an inhibitory effect of Dz22 on cell proliferation (Table 2).

Table 3
Cell cycle analysis of CasKi cells

	Percentage of G1 Phase Cells (%)				
Treatment	Expt 1	Expt 2	Expt 3	Expt 4	Average
No DNAzyme	43.54	51.42	46.77	56.33	49.54
Random	45.42	52.82	53.54	57.27	52.26
Anti-E6 DNAzyme	50.77	54.45	57.10	60.69	55.75

References

Barry, Ed. (1983) Formulation of Dermatological Vehicles, pp. 296-350; Marcel Dekker, Inc., New York and Basel.

Breaker, R.R. and Joyce, G. (1994) Chemistry and Biology 1:223-229.

Breaker, R.R. and Joyce, G.F. (1995) Chem. & Biol. (2):655-600.

Carmi, N., et al. (1996) Chemistry and Biology 3:1039-1046.

Cowsert, L.M., et al. (1993) Antimirobial Agents and Chemotherapy 37:171-177.

Crook, T., et al. (1991) Cell 67:547-556.

Dollard, S.C., et al. (1993) J Virol. 67:1721-1726.

Dyson, N., et al. (1989) Science 243:934-937.

Haseloff, J., Gerlach, W.L. (1988) Nature (334):585-591.

Hu, G.Y., et al. (1995) Cancer Gene Ther. 2:19-32.

Kashani-Sabet, M., et al. (1992) Antisense Research and Development 2:3-15.

Koizumi, M., et al. (1989) Nucleic Acids Research 17:7059-7069.

Martindale's Extra Phamacopoeia, 31st Ed. (1996).

Morris, J.D.H., et al. (1993) Oncogene 8:893-898.

Myers, G., et al., Eds. (1997) Human Papillomaviruses, Los Alamos Laboratory.

Nuovo, G.J., et al. (1990) Obstet Gynecol 75:1006-1011.

Otsuka, E. and Koizumi, M., Japanese Patent No. 4,235,919.

Pan, T. and Uhlenbeck, O.C. (1992) Biochemistry 31:3887-3895.

Phelps, W.C. and Alexander, K.A. (1995) Ann. Intern. Med. 123:368-382.

Ramchandani, M. and Toddywala, R. (1997) Formulation of Topical Delivery Systems, pp. 539-578; Eds. Ghosh and Pfister; Interpharm Press, Inc., Buffalo Grove, Illinois.

Raillard, S.A. and Joyce, G.F. (1996) Biochemistry 35:11693-11701.

Santoro, S.W., Joyce, G.F. (1997) Proc. Natl. Acad. Sci. (USA) 94:4262-4266.

Sato, H., et al. (1989) Virology 170:311-315.

Scheffner, M., et al. (1990) Cell 63:1129-1136.

Shillitoe, E.J., et al. (1994) Cancer Gene Ther. 1:193-204.

Sun, L.Q., et al. (1995a) Proc. Natl. Acad. Sci. (USA) 92:7272-7276.

Sun, L.Q., et al. (1995b) Nucleic Acids Res. 15:2909-2913.

Sun, L.Q., et al. (1997) Molecular Biotechnology 7:241-251.

Symonds, R.H. (1992) Annu. Rev. Biochem. 61:641-671.

Tsang, J. and Joyce, G.F. (1994) Biochemistry 33:5966-5973.

U.S. Pharmacopeia, XXIII (1995).

Von Knebel Doeberitz, M., et al. (1992) Int. J Cancer 51:831-834.

Wagner, R.W. (1995) Nature Medicine 1:1116-1118.

Wenniger and McEwen (1997) International Cosmetic Ingredient Dictionary and Handbook, 7th Ed., Vol 1-3.

Werness, B.A., et al. (1990) Science 248:76-79.

zur Hausen, H. and de Villiers, E.M. (1994) Annu. Rev. Microbiol. 48:427-447.

What is claimed is:

1. A DNAzyme which specifically cleaves HPV mRNA, comprising

(a) a catalytic domain that has the nucleotide sequence GGCTAGCTACAACGA and cleaves mRNA at any purine:pyrimidine cleavage site at which it is directed,

(b) a binding domain contiguous with the 5′ end of the catalytic domain, and

(c) another binding domain contiguous with the 3′ end of the catalytic domain,

wherein the binding domains are complementary to, and therefore hybridize with, the two regions immediately flanking the purine residue of the cleavage site within the HPV mRNA, respectively, at which DNAzyme-catalyzed cleavage is desired, and wherein each binding domain is at least six nucleotides in length, and both binding domains have a combined total length of at least 14 nucleotides.

2. The DNAzyme of claim 1, wherein each binding domain is nine nucleotides in length.

3. The DNAzyme of claim 1, wherein the 3′-end nucleotide residue is inverted in the binding domain contiguous with the 3′ end of the catalytic domain.

4. The DNAzyme of claim 1 having the sequence TCCCGAAAGGCTAGCTACAACGAATTGCAGT.

5. The DNAzyme of claim 1, wherein the cleavage site is purine:uracil.

6. The DNAzyme of claim 1, wherein the cleavage site is located in a region of the mRNA selected from the group consisting of the initiation site, a 5' untranslated region, and a 3' untranslated region.

7. The DNAzyme of claim 1, wherein the mRNA encodes a protein selected from the group consisting of HPV proteins E5, E6 and E7.

8. A pharmaceutical composition for treating or inhibiting the onset of an HPV-related disorder, which comprises the DNAzyme of claim 1 and a pharmaceutically acceptable carrier suitable for topical administration.

9. The pharmaceutical composition of claim 8, wherein the pharmaceutically acceptable carrier is selected from the group consisting of a liposome and a transdermal enhancer.

10. A method for inhibiting the onset of an HPV-related disorder in a subject at risk of contracting the disorder, which comprises topically administering to the subject a prophylactically effective dose of the pharmaceutical composition of claim 8.

11. A method for treating an HPV-related disorder in a subject, which comprises topically administering to the subject a therapeutically effective dose of the pharmaceutical composition of claim 8.

12. The method of claim 10 or 11, wherein the HPV-related disorder is selected from the group consisting of cervical carcinoma, cervical dysplasia and genital warts.

FIG. 1

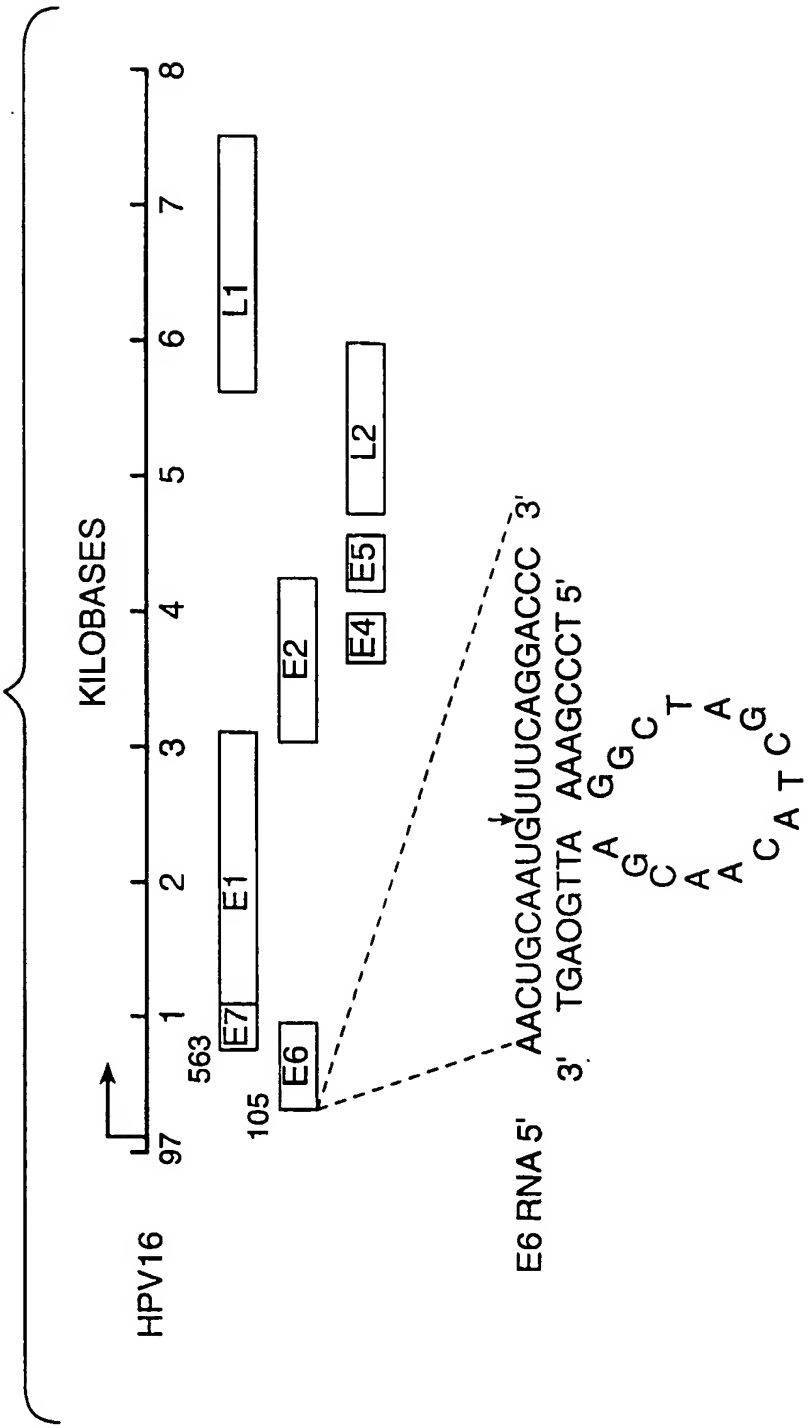

KILOBASES
1
2
3
4
5
6
7
8
HPV16
97
563
105
E7
E1
E6
E2
E4
E5
L2
L1
E6 RNA 5'
AACUGCAAUGUUUCAGGACCC 3'
3' TGAOGTTA AAAGCCCT 5'

FIG. 2

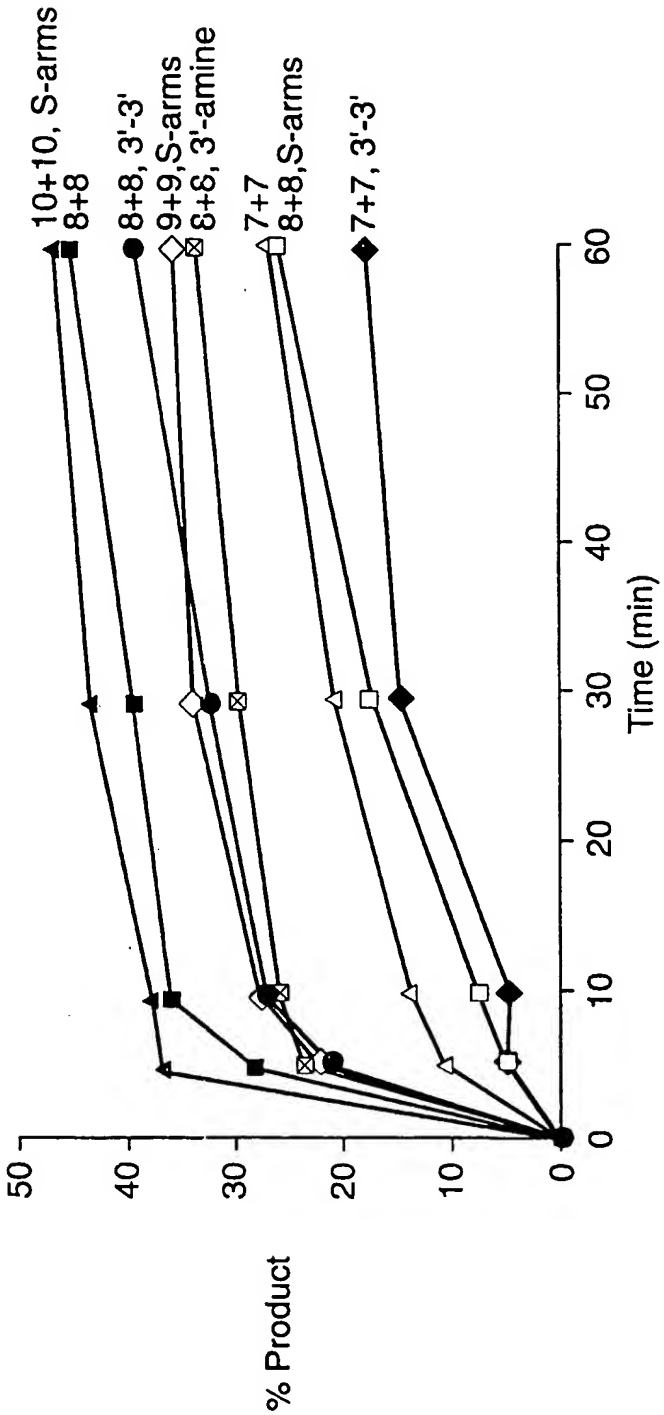

10+10, S-arms
8+8
8+8, 3'-3'
9+9,S-arms
8+8, 3'-amine
7+7
8+8,S-arms
7+7, 3'-3'
% Product
Time (min)
0
10
20
30
40
50
60

FIG. 3

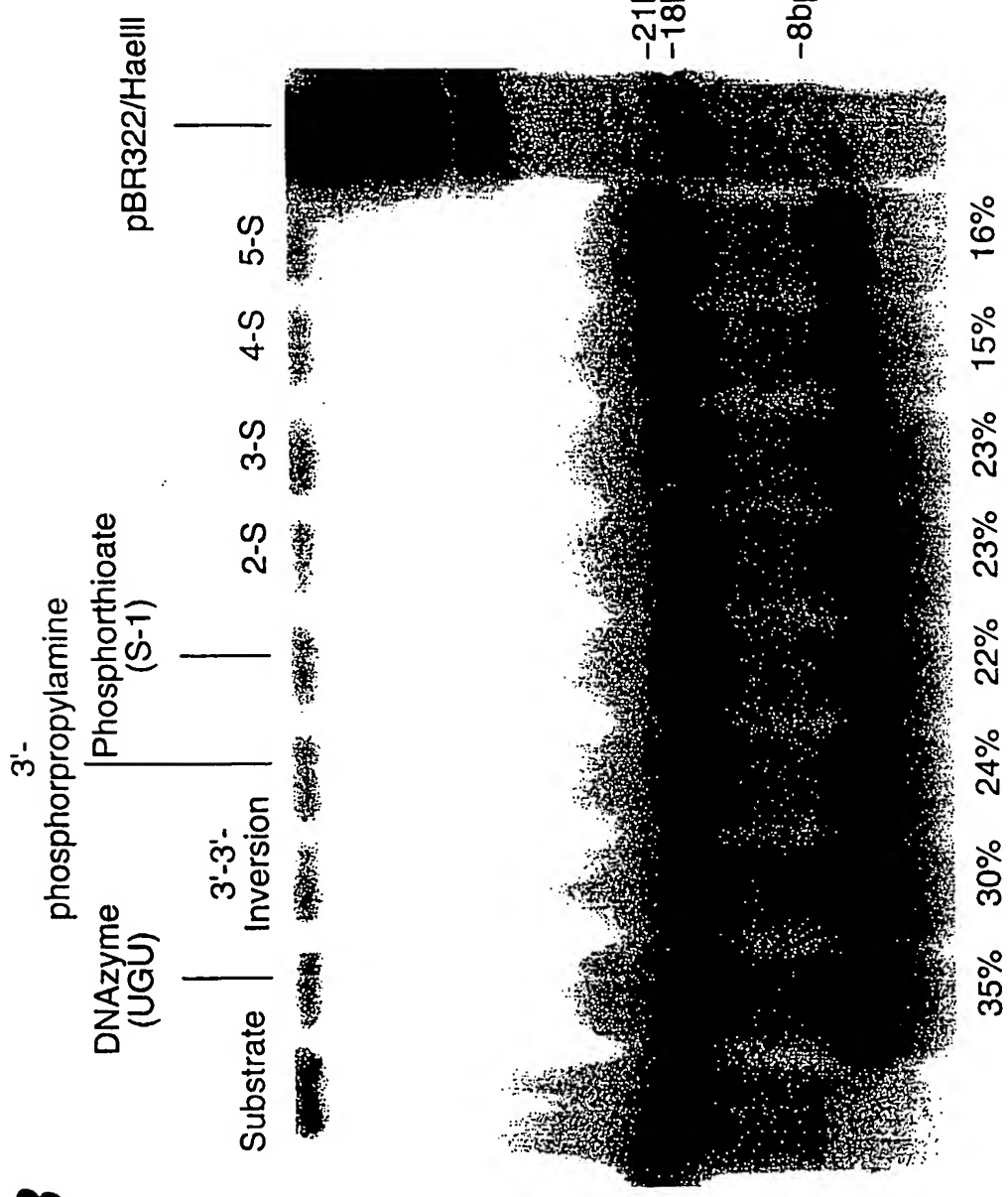


FIG. 4

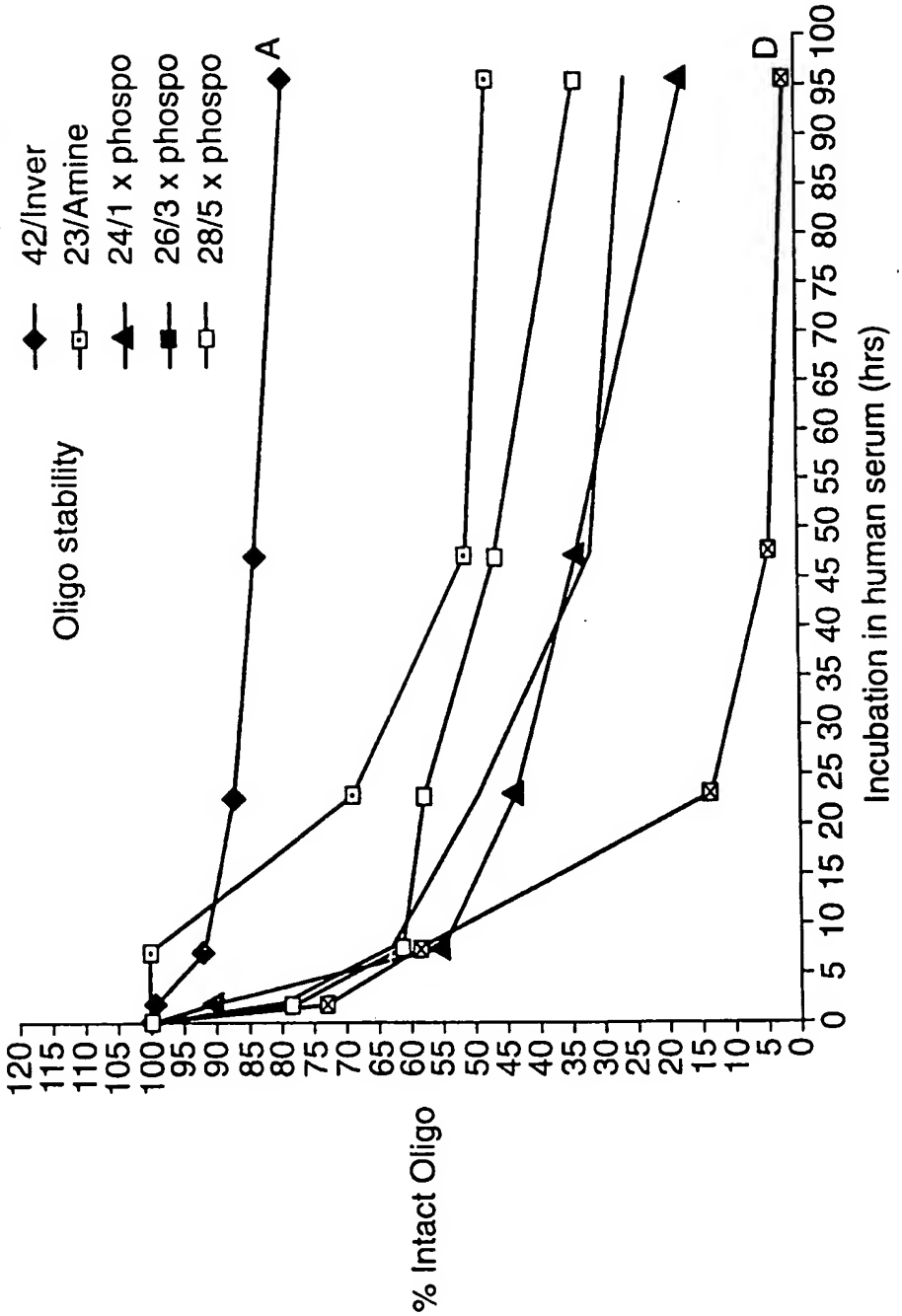

Oligo stability
41/Unmod
42/Inver
23/Amine
24/1 x phospo
26/3 x phospo
28/5 x phospo
A
D
% Intact Oligo
Incubation in human serum (hrs)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120

FIG. 5

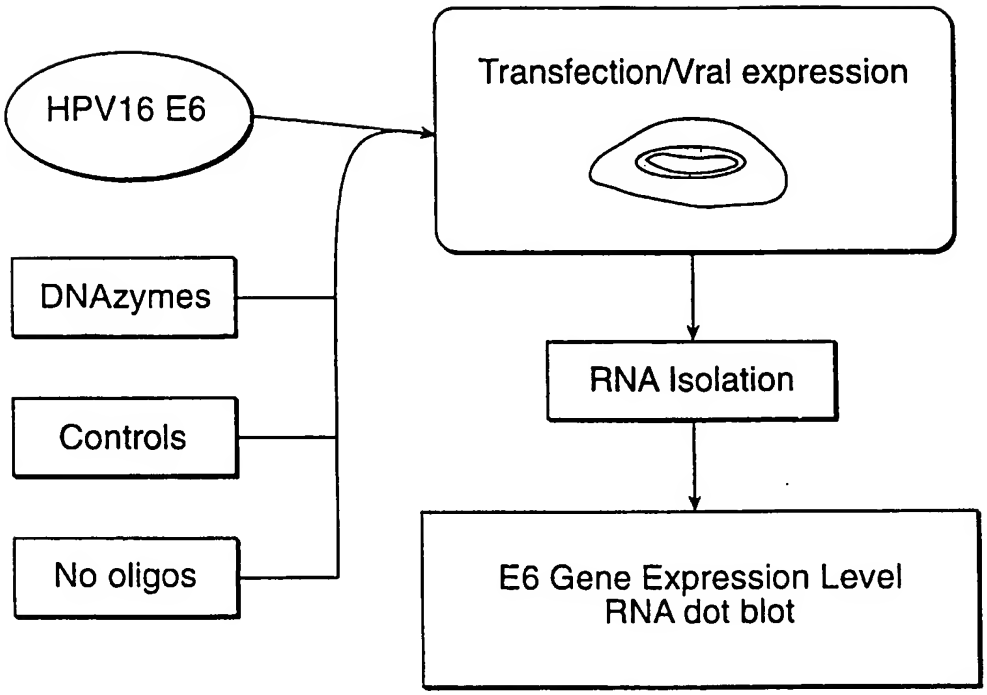
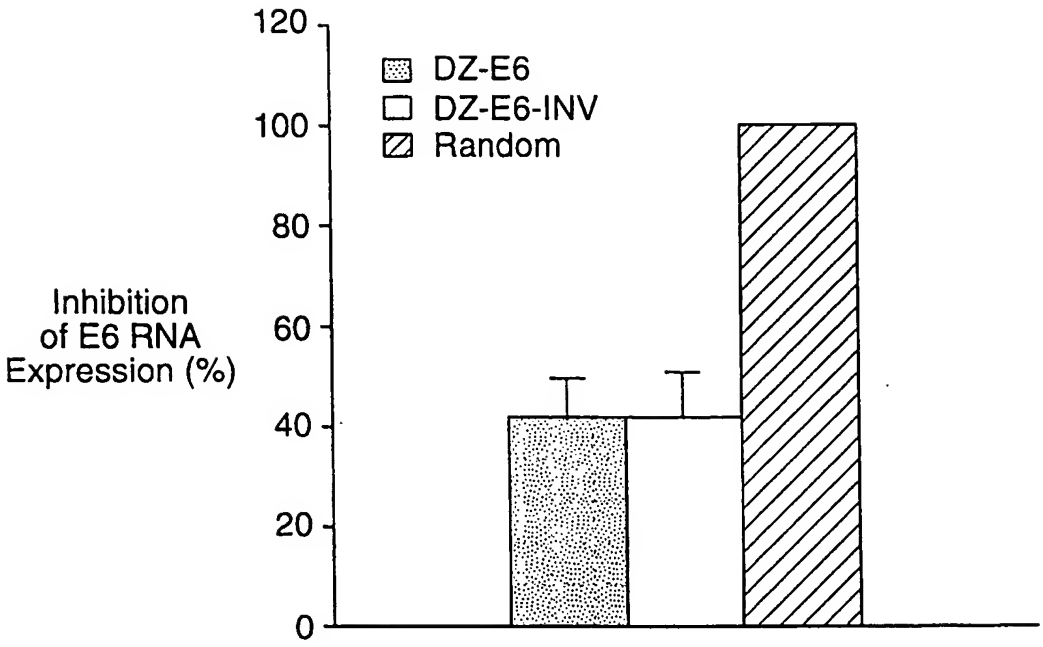

HPV16 E6
DNAzymes
Controls
No oligos
Transfection/Vral expression
RNA Isolation
E6 Gene Expression Level
RNA dot blot

FIG. 6


120
100
80
60
40
20
0
DZ-E6
DZ-E6-INV
Random
Inhibition of E6 RNA Expression (%)
Transient Assays for E6 DNAzymes (pooled)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 99/01486

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 9/16, A61K 38/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WPAT, CA: KEYWORDS (KW) See the electronic data base box below.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
MEDLINE, EMBL, GENBANK, DDBJ, PDB and Dgene (Derwent database)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DNA enzyme# or DNAzyme# or deoxyribozyme# or (catalytic DNA and (HPV or human papilloma virus)). Nucleic acid sequences: ggctagctacaacga, and tcccgaaaggctagctacaacgaattgcagt.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X P, Y	WO 98/49346 (THE SCRIPPS RESEARCH INSTITUTE) 5 November 1998 priority date 29 April 1997. See the whole document, especially pages 38, 95-98, sequence id no. 85 and figures 8-10	1-7. 8-12.
P, X	Cairns M. J. *et al* "Target site selection for an RNA-cleaving catalytic DNA" Nature Biotechnology vol 17 May 1999 pp 480-486. See the whole document.	1-12.

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 1999	15 DEC 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer J.H. CHAN Telephone No.: (02) 6283

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 99/01486

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	WO 96/17086 (THE SCRIPPS RESEARCH INSTITUTE) 6 June 1996. See the whole document, especially pages 11-12, 23, 51-54, sequence id no. 85 and figures 8-9	1-12.
X Y	Santoro S W and Joyce G F "A general purpose RNA-cleaving DNA enzyme" Proc Natl Acad Sci USA vol 94 pp 4262-4266 April 1997. See the whole document especially page 4264-6 and figure 2.	1-7. 8-12.
Y	Genbank accession no. g333031 publication date 18 March 1994.	1-7.
P, X P, Y	Santoro S W and Joyce G F "Mechanism and utility of an RNA-cleaving DNA enzyme" Biochemistry 1998 Sept 22, 37, 13330-42 See the whole document especially pages 13331, 13337-41 and figure 1	1-7. 8-12.
P, X	WO 99/50452 (JOHNSON & JOHNSON RESEARCH PTY LIMITED) 7 October 1999. See the whole document, especially pp 17-25.	1-12.
P,Y	Warashina M *et al* "Extremely high and specific activity of DNA enzymes in cells with a Philadelphia chromosome" Chemistry & Biology 1999 vol 6 pp 237-250. See the whole document especially figures 2, 4 and page 247.	1-12.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB 99/01486

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	98/49346	AU	72675/98				
WO	96/17086	AU	45950/96	BR	9510003	CA	2205382
		CN	1173207	EP	792375	FI	972333
		HU	77576	NO	972483	US	5807718
WO	99/50452	AU	35303/99				

END OF ANNEX